

***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***

Applicant: Peter BUDTZ, *et al.*  
Title: **ENZYMATIC PROCESS FOR OBTAINING  
INCREASED YIELD OF LACTOBIONIC ACID**  
Appl. No.: 10/572,911  
Examiner: Sheridan R. Macauley  
Art Unit: 1651  
Confirmation  
Number: 4642

DECLARATION UNDER 37 C.F.R. §1.132

The undersigned, Per Munk Nielsen, hereby declares as follows:

1. I received my M.S. degree, in Chemical Engineering, from Technical University of Denmark in 1979. I have been working in the fields of food science since 1980 and enzyme application developments since 1988.
2. Currently I am a Senior Science Manager at Novozymes A/S, with a facility located at Krogshøjvej 36, DK-2880, Bagsværd, Denmark. Novozymes A/S is a co-assignee of the above-referenced application.
3. At the present position, I have the responsibilities of development of enzyme applications within the Oils and Fats Industry.
4. I am a named inventor on the captioned application, which I have read and believe that I have understood.
5. Experiments were performed, by me or under my direction, to produce lactobionic acid by allowing a carbohydrate oxidase to convert lactose in the presence and in the absence, respectively, of a catalase and/or a base.

6. In the absence of a catalase or a base, the conversion rate of lactose to lactobionic acid was 41% (see Example 1 of the application), whereas, in the presence of a catalase, the conversion rate was increased to 100% (see Example 3). Unexpectedly, the presence of a catalase increased the conversion rate of lactose to lactobionic acid by about 144%.

7. The presence of both a catalase and a weak base not only increased the conversion rate but also reduced the reaction time from about 6.5 hours in the presence of a strong base (NaOH), to about 4.5 hours in the presence of a weak base ( $\text{Na}_2\text{CO}_3$  or  $\text{NH}_3$ ). See Example 5 of the application. Surprisingly, about a 32% reduction in reaction time was effected by adding a weak base rather than a strong base.

8. Achieving both increased conversion rate and reduced reaction time when a catalase was added with a weak base but not with a strong base was unexpected because no principal or principals known at the time reasonably predicted this result *a priori*. This fact is evidenced in the acceptance for publication, by a peer-reviewed journal, of a report that I co-authored on the above-described experiments. See Nordkvist *et al.*, *Biotechnology and Bioengineering* 97: 694 (2006), a copy of which is appended to this declaration.

9. I declare that the statements made herein of my knowledge are true and all statements on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therein.



Per Munk Nielsen

March 31, 2011

Date